Doc.Ref.: PE – 006 – PSA/BM

Contagious bovine peripneumonia (PPCB) - Complement Fixation Test

Principles of Good Laboratory Practice

- Complement Fixation (FC) test is used to detect and quantify antibody in serum that does not form visible precipitate or agglutinate when reacted with antigen until complement is used.
- The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane. Complement can only bind antigen-antibody (Ag-Ab) complexes.
- Antigen-antibody complex fixes the complement. But the fixation of complement with Ag-Ab complex does not have any visible effect like agglutination and precipitation. So, it is necessary to use indicators system. The indicator system consists of sheep RBC coated with anti-sheep RBC antibody. If the antibody is bound to antigens adsorbed on red blood cells, they are destroyed and haemolysis occurs.
- The CF test can be a qualitative or semi-quantitative test, when a serial dilution of the sample is performed, observing the maximum dilution where a positive reaction occurs.
- The quality control and standardization of all reagents is a critical point in CF test harmonization. Thus, it is important to use appropriate controls, which should be requested from the OIE Reference Laboratories for the PPCB.
- The characteristics of all reagents used must be taken into account, as they have an impact on the interpretation of the test result. It is essential to control the origin and validation dates of all reagents.
- The quality of the water used for diluting reagents (veronal buffer, complement, amboreceptor) must be controlled. Preferably use ultrapure water, with pH 6.5 ± 0.2, resistivity of 18.2 MΩ / cm and maximum conductivity of 0.1 µS / cm at 25ºC.
- Check room temperature. Only start the test with a temperature between 18 and 26ºC.
- Samples and reagents must be at room temperature (22±4ºC) at the beginning of the test.
- Inactivation of serum samples is important to reduce bacterial contamination, to destroy the natural complement of the serum itself, to reduce most non-specific IgM. Inactivation must be carried out on the same day; if the test must be repeated in the next day, the serum sample should be inactivated before the test for only 10 minutes. Inactivation according to:
    - Diluted sera - inactivation at 58±2 ºC for 30-50 minutes
    - Undiluted sera - inactivation at 60±2 ºC for 30 minutes